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Elevation of truncated (48 kDa) form of unconventional myosin 1C in blood serum correlates with severe Covid-19

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ABSTRACT

In Covid-19 and autoimmune patients, there are several similarities revealed in the immune responses (Liu et al., 2021; Woodruff et al., 2020). Earlier, we firstly detected a truncated (48 kDa) form of the unconventional Myosin 1C (48/Myo1C) in a fraction of proteins soluble in 10% 2,2,2-trichloroacetic acid (TCA). These proteins were obtained from blood serum of patients with autoimmune diseases, such as multiple sclerosis, systemic lupus erythematosus, and rheumatoid arthritis (Kit et al., 2018). Here, we demonstrated that content of 48/Myo1C was also elevated in blood serum of the severe Covid-19 patients. Whereas in blood of 28 clinically healthy human individuals regularly tested for Covid-19 infection, the amount of this protein was undetectable or very low, in blood of 16 of 28 patients hospitalized with severe course of this disease, its amount was significantly increased. Dexamethasone, steroid hormone which is widely used for treatment of severe Covid-19 patients, induced time-dependent elevation of the 48/Myo1C in blood of such patients. The 48/Myo1C dose-dependently suppressed the viability of anti-CD3-activated lymphocytes of human peripheral blood. Recently, we used affinity chromatography on the magnetic poly(glycidyl-methacrylate) (mag-PGMA–NH_2) microparticles functionalized with Myo1C and MALDI–TOF mass spectrometry with molecular modeling *in silico* in order to identify potential molecular partners of the 48/Myo1C. It was found that 48/Myo1C might bind to component 3 of the complement system and the anti-thrombin-III (Starykovych et al., 2021). Thus, the mechanisms of the pathogenic action of truncated form of Myo1C in severe COVID-19 patients may involve a suppression of the immune cells, as well as modulation of complement and coagulation cascades.

1. Introduction

The intensive inflammatory processes aimed at suppression of SARS-CoV-2 virus lead to serious destructive events in the organism of severe Covid-19 patients. To fight the virus, cells of the immune system produce huge amount of pro-inflammatory molecules through a phenomenon known as “cytokine storm”. Antibodies are also produced triggering blood clotting and inflammation in multiple organs, tissues and blood vessels (Woodruff et al., 2020).

It was found that in Covid-19 and autoimmune patients the immune responses have several similarities (Liu et al., 2021; Woodruff et al., 2020). A search for the biomarkers of such similarities and defining their role in the destructive processes observed in severe Covid-19 patients are of great significance for better understanding mechanisms of Covid-19 pathogenesis. Earlier, we firstly described a 48 kDa fragment of the unconventional myosin 1C (48/Myo1C) in blood serum of patients with various autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus (Kit et al., 2018). Here, we

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carried out a search for the 48/Myo1C in blood serum of Covid-19 patients with severe forms of this disease. We have detected this product of degradation of the Myo1C in the severe Covid-19 patients and showed that its level in those patients was significantly higher than in healthy human individuals. Recently, we revealed a capability of binding of 48/Myo1C to component 3 of the complement system and to anti-thrombin III of the coagulation cascade (Starykovych et al., 2021). Thus, the pathogenic action of 48/Myo1C can involve complement system and coagulation cascade that are heavily disturbed in patients with severe Covid-19. Here, we also described a suppression of the immune cells by the 48/Myo1C, the ways of its possible generation, and the regulation of its content in the severe Covid-19 patients by the corticosteroid hormone.

2. Materials and methods

2.1. Samples of human blood serum

Peripheral venous blood was obtained from 28 human individuals without clinical manifestations of Covid-19 and 28 patients with Covid-19 who were hospitalized because of a severe course of this disease. Serum was obtained after blood coagulation for 30 min at 23 °C, followed by centrifugation for 10 min at 5000*g*. For verification of Covid-19 diagnosis, samples of blood serum were tested in clinical diagnostic laboratory with Vitrotest® SARS-CoV-2 IgG and Vitrotest® SARS-CoV-2 IgM Kits (“Vitrotest®”, Ukraine). Blood samples were collected under the approval of the Bio-Ethics Review Board at the Danylo Halytsky Lviv National Medical University in accordance with the recommendations of the Ministry of Health of Ukraine and statements of National Bioethics Advisory Commission for research involving human biological materials: issues and policy guidance (1999), available at <www. bioethics.georgetown.edu/nbac>.

2.2. Experiments with dexamethasone treatment

Severe Covid-19 patients were injected with dexamethasone (4 mg/ml, KRKA representative in Ukraine) daily (1 ml) for 4 days. Venous blood was collected, as described in the Sub-chapter 2.1.

Treatment of laboratory rats. Three non-linear white rats, 240 g each, were injected with methyl-prednisolone (purchased from Pfizer distributor in Ukraine) daily (1 mg/kg/day per animal) for 4 days. Blood was collected by puncture of the retro-orbital plexus every 24 h for 4 days. Once the blood clot was formed, serum was collected and immediately stored at -20 °C.

2.3. Isolation of human blood lymphocytes and their activation

Twenty milliliters of venous blood were taken from human volunteers (Approved by the Bio-Ethics Committee of the Institute of Cell Biology, NAS of Ukraine, Protocol No 2 dated by 27.01.2019) and mixed with 200 μl of heparin. Fresh blood was diluted 2-fold with 0.9% NaCl under sterile conditions. Isolation of lymphocytes was performed in a density gradient of ficol-verografin using protocol of the manufacturer (Lympoprep, NYCOMED PHARMA AS, Oslo Norway). The resulting lymphocytes were re-suspended in the RPMI-1640 medium, and cultured for up to 10 days. To separate the lymphocytes from the monocytes, cell suspension was left for 24 h. After 24 h of culture, the monocytes were attached while the lymphocytes were transferred to a fresh Falcon tube (15 ml). Stimulation of growth and proliferation of lymphocytes was achieved during their culturing for 24 h on plastic plates coated with anti-CD3 antibody in the RPMI-1640 medium (Sigma, USA) supplemented with 20% fetal bovine serum (FBS) (Sigma, USA). Cells were kept in the CO_2-incubator at 37 °C, 5% CO_2 and 95% humidity in accordance with experiment protocol. After that, lymphocytes were treated with 48/Myo1C for 24 or 48 h.

2.4. UV-irradiation of human blood lymphocytes

1×10^6 human lymphocytes were washed with sterile 1× PBS and irradiated for 2 min in the MacroVue UV-20 trans-illuminator (302 nm filter, Hoeffer, USA). After such treatment, cells were incubated for 2 h at 37 °C at 5% CO_2 and pelleted by centrifugation at 1000 rpm for 5 min. Cell morphology was analyzed at 400× magnification under Mik-Med-12 microscope (LOMO, St. Petersburg, Russian Federation). Cells were stained with fluorescent dyes: Hoechst 33342 (final concentration - 0.5 μg/ml, Sigma, USA) and Propidium iodide (final concentration – 0.5 μg/ml, Sigma, USA). Cell morphology was studied under the microscope at the 320–390 nm excitation wavelength and at 420–480 nm emission wavelength. Cells were lysed with a solution of 1% Triton X-100 in 20 mM Tris-HCl in the presence of 1 mm phenylmethanesulfonyl fluoride (PMSF, Roche, Basel, Switzerland). The lysates were dissolved in the denaturing buffer (1% SDS, 20 mM Tris-HCl, pH 6.8). Proteins were separated by the SDS-PAGE in 12% polyacrylamide gel. The proteins on the gel was stained with Coomassie BBR-100 Blue dye (Giottobiotech, Italia).

2.5. Evaluation of cytotoxic effect of studied substances

For *in vitro* screening of cytotoxic action of the 48/Myo1C, MTT-test based on cell staining with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide dye (Sigma-Aldrich, USA) was applied. Lymphocytes were seeded for 24 h in 96-well plates in 100 μl at a concentration of 10^5 cells/well. After that, lymphocytes were incubated with various agents for the next 48 h. MTT was added to these cells according to the manufacturer’s protocol (Sigma-Aldrich, USA). The purple product (formazan crystals) of the MTT reaction was dissolved in the DMSO (CARLO ERBA Reagents S.A.S, France) and optical density was measured at 630 nm with an Multiplate Reader BioTek ELx800 (BioTek Instruments, Inc., USA).

2.6. Isolation of 48/Myo 1C from human blood serum

Two hundred μl of blood serum was diluted 2-fold with the phosphate buffered saline (PBS), after which 100% TCA was added to 10% final concentration. After 30 min incubation on wet ice, samples were centrifuged for 15 min at 10,000 g. 200 μl of the supernatant containing TCA-soluble compounds were transferred to a fresh Eppendorf tube (1.5 ml), and cold (−20 °C) acetone was added to a final volume of 1.5 ml. The obtained mixture was kept at −20 °C for 18 h and the precipitate was pelleted by centrifugation at 10,000 *g* for 30 min. The pellet was dried at 37 °C, diluted in 30 μl of with Sodium dodecyl sulphate (SDS)-containing buffer, boiled for 5 min, and samples were stored at −20 °C until use, but no longer than 2 weeks. The obtained samples were subjected to the SDS-PAGE in 12% polyacrylamide gel (Laemmli, 1970), followed by protein staining in gel with Coomassie brilliant blue G-250 (Sigma-Aldrich, USA). The individual electrophoretic bands of proteins were excised from the gel and subjected to in-gel digestion with trypsin (Promega, USA), followed by the mass spectrometry (see **2.8**).

2.7. Western-blot analysis of cellular proteins

Proteins were extracted from the mononuclear cells of human peripheric blood with lysis buffer (20 mM Tris-HCl, pH 8.0, 1% Triton-X100, 150 mM NaCl, 50 mM NaF, 0.1% SDS) containing 1 mM PMSF and 10 μg/ml of cocktail of protease inhibitors “Complete” (Roche, Basel, Switzerland), subjected to electrophoresis in 12%–15% SDS-PAGE, and transferred onto the nitrocellulose membrane (Schleicher and Schuell, Germany). The membrane was blocked with 3% BSA for 1 h at 23 °C, washed with the PBST buffer (PBS supplemented with Tween-20) (three times for 5 min of each wash), and incubated overnight with primary human antibody (Myo1C, AVIVA SYSTEM BIOLOGY, No ARP56292), 0.1 μg/ml in 5% bovine serum albumin (BSA)-supplemented with the

PBST. After incubation, the blotted membrane was washed with the PBST (three times for 5 min each wash), and incubated for 1.5 h with horseradish peroxidase-conjugated anti-mouse IgG antibody (**Cell Signaling Technology Europe**, the Netherlands). Proteins on the blot were visualized with the ECL reagent (Sigma, USA).

2.8. Mass spectrometry confirmation of 48 Da/Myo1C

The protein bands were subjected to in-gel digestion with trypsin (sequencing grade, Promega, USA), as described earlier (see: **2.7**). Generated peptides were subjected to a solid phase extraction and cleaning with use of ZipTip C18 (Millipore, USA). The obtained peptides were directly injected in Agilent 6546, Q-TOF for MS/MS analysis. MS/MS mass spectra were collected in an automated mode. Significance of searches was $p < 0.05$, delta tolerance 1.2 Da for the precursor ions and 0.6 Da for MS/MS fragments. Identification was repeated in six independent experiments. Generated mass spectra were searched in Mascot (Matrix Sciences, USA) for identification. The sequences of 2 peptides confirmed identification of the protein as a 48 kDa fragment of unconventional myosin 1C.

2.9. Statistical analysis

All experiments were repeated three times with three parallel repeats. The Analysis of Variance (ANOVA) was used as a statistical test for the comparison of the experimental groups. Two-way ANOVA with Bonferroni posttests was applied in order to compare replicated means by rows using GraphPad Prismv6.0 software.

3. Results and discussion

Monitoring of Covid-19 biomarkers is of critical significance for the establishment of the disease severity and evaluation of an efficiency of its treatment and prognosis (Fei et al., 2020, 6). It was found that the medications used to treat patients with autoimmune diseases of the rheumatologic etiology also possessed therapeutic effects in the severe Covid-19 patients which suggests the interrelationships in pathogenesis of these pathologies (Liu et al., 2021).

In a search for novel blood serum biomarkers of Covid-19 severity, a novel procedure was developed by us for isolation of blood serum proteins soluble in 10% TCA. Traditionally, it is considered that all proteins of high and middle size molecular weight are pelleted with 10% TCA. However, unexpectedly, a significant amount of 48 kDa protein was detected at the SDS-PAGE electrophoresis with regular Coomassie G-250

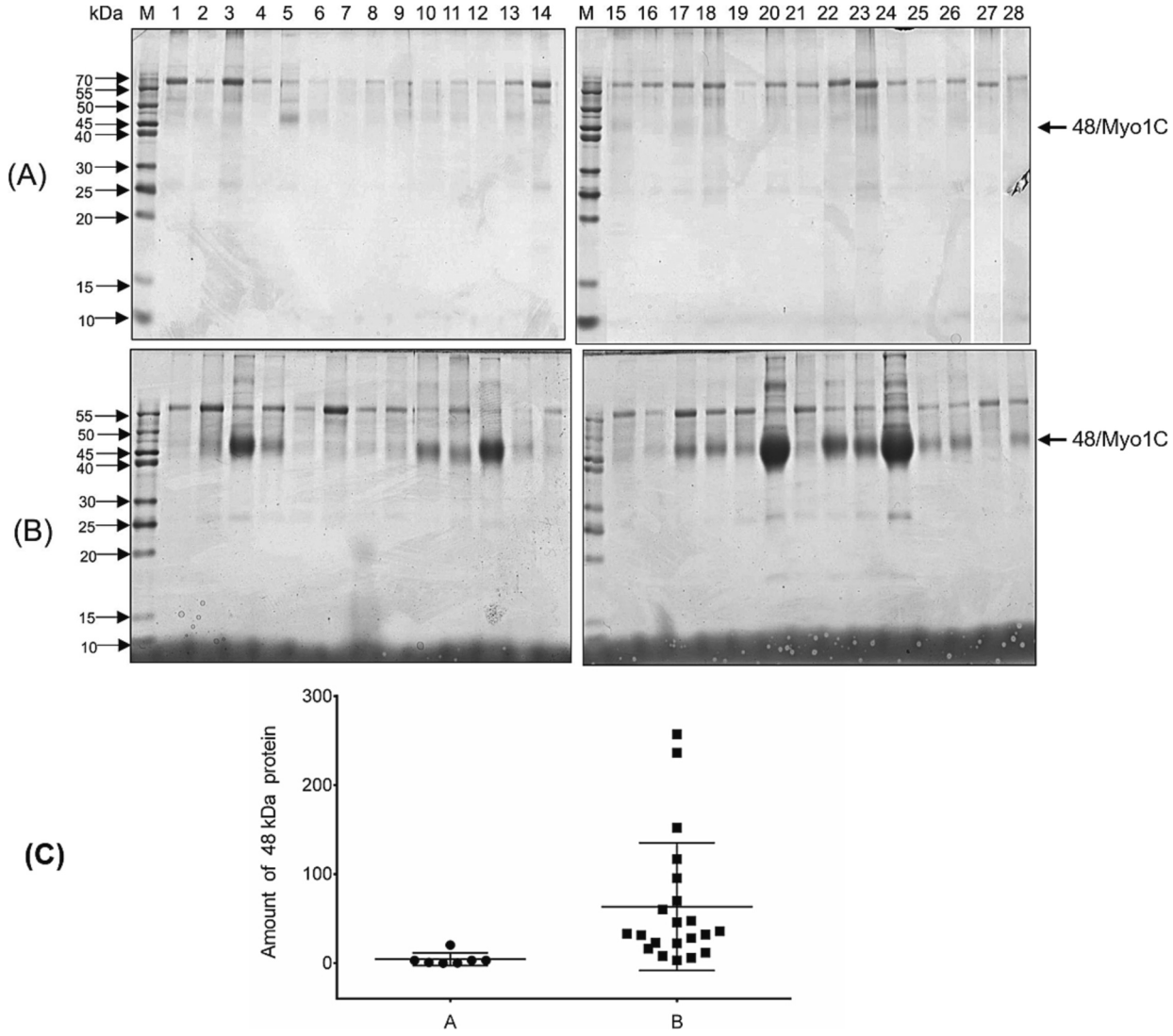

Fig. 1. Electrophoretic (SDS-PAGE) pattern of 10% TCA-soluble proteins isolated from blood serum of 28 clinically healthy human individuals (A) and of 28 severe Covid-19 patients (B). Results of densitometric analysis of the electrophoretic data (C).

staining of proteins extracted with 10% TCA from blood serum of patients diagnosed for the autoimmune diseases, such as multiple sclerosis, systemic lupus erythematosus and rheumatoid arthritis (Starykovych et al., 2021), as well as in patients diagnosed for the non-Hodgkin's lymphoma and multiple myeloma (Myronovskij et al., 2017). MALDI TOF/TOF mass-spectrometry combined with the bio-informatics analysis permitted us to identify that protein as a 48 kDa fragment of the unconventional Myo1C (Kit et al., 2018).

In Fig. 1, the electrophoretic patterns of 10% TCA-soluble proteins isolated from blood serum of 28 clinical healthy human individuals (A) and of 28 severe Covid-19 patients (B) are presented (Orfin et al., 2021). These patterns demonstrate an elevated amount of 48 kDa protein in blood serum of 16 among 28 patients with severe Covid-19, compared to a lack of this protein in blood serum of healthy individuals. Different levels of 48 kDa protein in a cohort of the severe Covid-19 patients (Fig. 1, C) suggest a variability in the expression of this protein in these patients. As it was earlier shown by us (Myronovskij et al., 2015), protein band of approximately 66 kDa that is present in practically all samples under study belongs to human blood serum albumin which is very difficult to avoid in samples isolated from blood.

Mass spectrometry was applied to confirm that 48 kDa protein detected in blood serum of severe Covid-19 patients is really a truncated form of the Myo1C. Initial identification was performed by the MALDI-TOF mass spectrometry, and a confirmation was conducted by the MS/MS sequencing of peptides (see: Supplementary materials). Two selected peptides confirmed the Myo1C sequence. The MS/MS analysis confirmed that the protein band present in Fig. 1 is a 48 kDa fragment of the unconventional myosin 1C. We have carried out identification of proteins in two electrophoretic bands of samples obtained from two Covid-19 patients who were hospitalized due to a severe course of this disease.

Taking into account our results about the elevation of the 48/Myo1C in blood serum of severe Covid-19 patients, it was reasonable to investigate the dynamics of its content during cure of these patients with dexamethasone, the corticosteroid hormone that is widely used for treatment of the severe Covid-19 patients (Liu et al., 2021, Mehta et al., 2022. It was found that the dynamics of the 48/Myo1C content in blood of the treated patients is characterized by two phases, namely, its gradual increase (24–72 h after 1st injection), followed by its sharp decrease (96 h) (Fig. 2). Corticosteroids are known to be used to suppress the development of the acute phase of the autoimmune diseases, when these hormones may cause a destruction of the autoimmune cells. Thus, it is possible that a generation of the truncated form of Myo1C takes place in that period, since this protein was shown to be abundant in the immune B-cells (Maravillas-Montero et al., 2011, Maravillas-Montero et al., 2014. As one can see in Fig. 2, second phase observed in the expression of the 48/Myo1C in blood serum of the dexamethasone-treated severe Covid-19 patients is characterized by a sharp decrease in the amount of this protein. One may speculate that such a decrease in the 48/Myo1C level demonstrates a development of favorable events in the Covid-19 patients accompanied by a suppression of the destructive processes observed during a "cytokine storm" (Woodruff et al., 2020).

It should be noted that we detected almost the same time-dependent dynamics of the 48/Myo1C content in blood serum of corticosteroid-treated laboratory rats (Fig. 3). As in Covid-19 patients, two phases in its content were observed, namely a gradual increase of its content during 24–72 h after the 1st injection of steroid hormone, followed by a sharp (6.5-times) decrease in its content at 96 h time point, comparing to 72 h time point.

Recently, we started a search for the potential molecular partners of the 48/Myo1C in human organism. To do that, we immobilized 48/Myo1C on the monodisperse magnetic poly(glycidyl methacrylate) (mag-PGMA-NH_2) microspheres and used these microspheres for affinity chromatography of blood serum proteins of the multiple sclerosis patients. The results of the conducted Western-blot analysis of the absorbed proteins demonstrated that IgG and IgM, but not IgA, were the affinity partners of the 48/Myo1C (Horák et al., 2017; Zasońska et al., 2018). Thus, the anti-Myo1C autoantibodies of the IgG and IgM classes found in blood serum of the multiple sclerosis patients could be also generated in the severe Covid-19 patients and present in their blood serum.

In the severe Covid-19 patients, the extrafollicular B cell activation, previously revealed in the autoimmune patients, was detected (Woodruff et al., 2020). Such activation correlated with an expansion of the antibody-secreting cells and production of antibodies neutralizing SARS-CoV-2. Since in those patients, the disease demonstrated severe manifestations, such as strongly enhanced inflammation, multi-organ impair and even death, an extreme immune activation might play a pathologic role in the severe Covid-19 patients. In these patients, the extrafollicular B cells, similar to B cells found in active lupus, a known autoimmune disease, have been identified. In the severe Covid-19 patients, autoantibodies typical for autoimmune disorders such lupus can be produced in addition to protective anti-viral antibodies. Instead of suppressing virus or other pathogens, these antibodies may attack some human tissues.

Next, we attempted to define a source of generation of the 48/Myo1C and its potent biological actions. It was shown that the Myo1C is abundant in B lymphocytes (Maravillas-Montero et al., 2011; Maravillas-Montero et al., 2014). That is why, we applied Western-blot

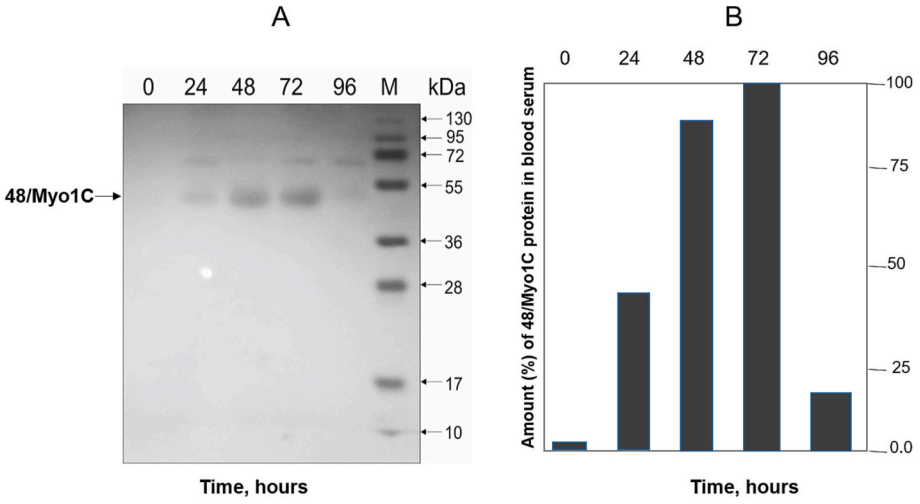

Fig. 2. Changes in the 48/Myo1C level in blood serum of human at 4 day treatment with a single daily dose (4 mg/ml, 1 ml) of dexamethasone. A - SDS-PAGE-electrophoresis of 10% TCA-extracted proteins. B – Results of densitometric analysis of the electropherogram.

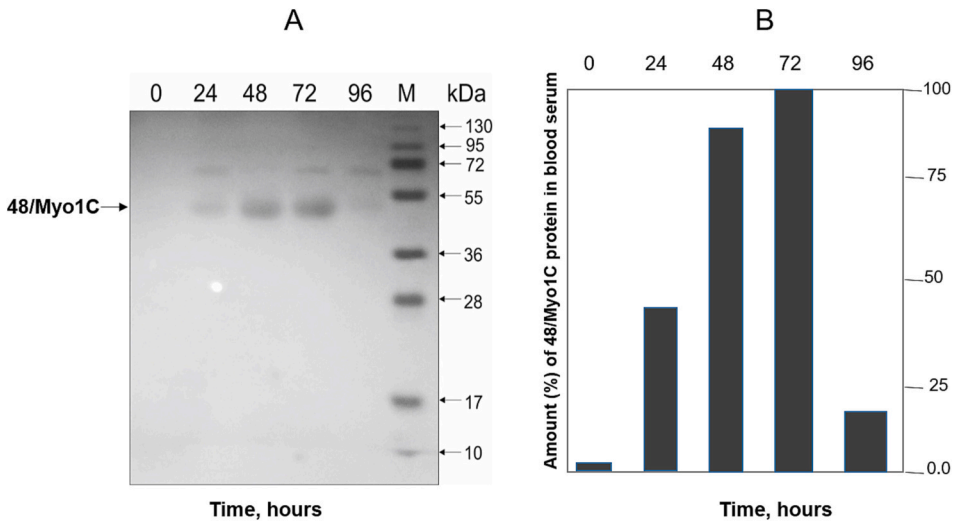

Fig. 3. Dynamics in the level of the 48/Myo1C in blood serum of laboratory rats at 4 day treatment with methyl-prednisolone (1 mg/kg/day per animal). A - SDS-PAGE -electrophoresis of 10% TCA-extracted proteins. B - Results of densitometric analysis of the electropherogram.

analysis with monospecific anti-Myo 1C antibodies in order to detect proteins with affinity to the Myo1C towards the mononuclear cells (mostly lymphocytes) of human peripheric blood. These cells were subjected to UV-radiation which is known to induce apoptosis in the lymphocytes (Artiukhov et al., 2012). In the lysate of proteins of the intact (untreated lymphocytes), there was only one band of m.m. 121 kDa with the affinity to the anti-Myo1C antibodies (Fig. 4) that corresponded to the Myo1C (https://www.uniprot.org/uniprot/O00159). Whereas the electrophoretic pattern of proteins detected in the lysate of the UV-treated lymphocytes contained several other protein bands

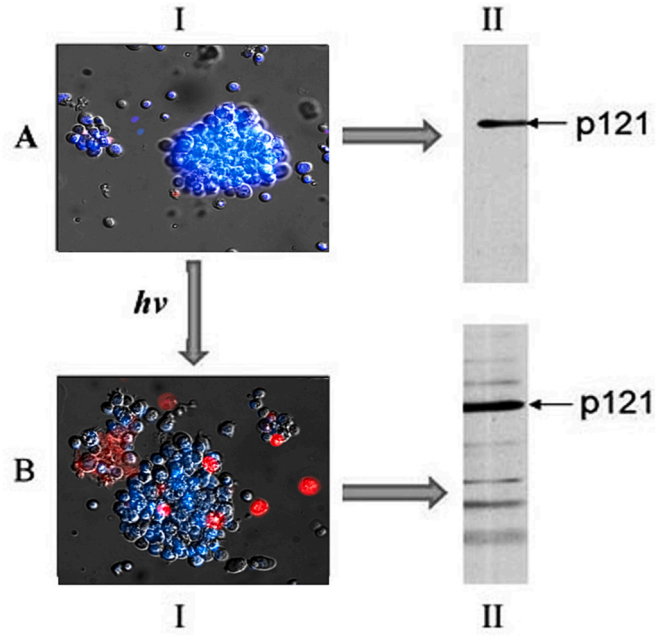

Fig. 4. Effect of the UV-irradiation on the pattern of molecular forms of the Myo1C in human peripheral lymphocytes before (A) and after (B) UV-irradiation. I - fluorescent microscopy of lymphocytes. II – Results of Western-blot analysis of cell lysates using commercial monospecific anti-Myo1C antibodies. Lymphocytes were stained with Hoechst 33342 (blue fluorescence) and propidium iodide (red fluorescence). Staining of cells with the propidium iodide is an indicator of plasma membrane damage leading to cell death. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

recognized with anti-Myo1C antibodies, in addition to the main band of native Myo1C (Fig. 4). Thus, a destructive (pro-apoptotic) action of the UV-radiation towards lymphocytes of the peripheric blood was accompanied by the proteolytic degradation of the Myo1C and generation of the 48/Myo 1C which, in turn, inhibits the viability the immune cells. There are numerous publications showing the hyperproduction of the proinflammatory cytokines in the Covid-19 patients (Fathi and Razaei, 2020). Such a “cytokine storm” leads to multi-organ injury and also induces apoptosis of the immune cells resulting in the lymphocytopenia whose degree correlates with Covid-19 severity (Fei et al., 2020).

The Myo1C belongs to unconventional myosins of vertebrates and can interact with the microfilament cortex of plasma membrane. The myosins of this type participate in regulation of various functions, such as cytoskeleton organization, cell motility, and even nuclear transcription (Calluccio, 2018). The Myo1C is present in membrane microvilli of B lymphocytes where it participates in the cytoskeleton dynamics and sorting of B lymphocytes (Maravillas-Montero et al., 2011; Maravillas-Montero et al., 2014).

There is no reliable information regarding the biological functions of a 48 kDa fragment of the unconventional myosin 1C. Its role as a diagnostic and prognostic biomarker of the severity of the disease was shown in the multiple sclerosis patients (Nehrych et al., 2018). Here, we prepared primary culture of the anti-CD3 antibody-activated lymphocytes of the peripheral blood of healthy human and treated these cells with the 48/Myo1C. It was found that the 48/Myo1C inhibited the viability of these immune cells in time dependent manner (Fig. 5). Thus, this effect could be a mechanism of the 48/Myo1C-related immune suppression in the autoimmune patients.

Summarizing, cells which interact with Sars-CoV2 can be divided in two main categories: 1) *cells which fight with the infection* (various immune cells, mainly, T cells (Moss, 2022), NK cells (Krämer et al., 2021) and memory B cells (Muecksch, 2022); 2) *cells which are the “victims” of Sars-CoV2* infection and can be detected due to co-localization of the ACE2 receptor and viral antigen(s). Such a co-expression demonstrated that SARS-CoV-2 triggered multi-organ impair that involved lung and trachea, small intestine and pancreas, kidney and liver, heart, blood vessels and endothelial system (Michael et al., 2022). An acute respiratory distress syndrome belongs to the heaviest complications detected in the Covid-19 patients. It may lead to sepsis which correlates with the auto-immune response in these patients (Juanes-Velasco et al., 2022).

The pattern which we detected in time-dependent dynamics of the 48/Myo1C content (Figs. 2 & 3) may suggest an existence of an “acute” phase characterized by the elevation of the 48/Myo1C content in blood

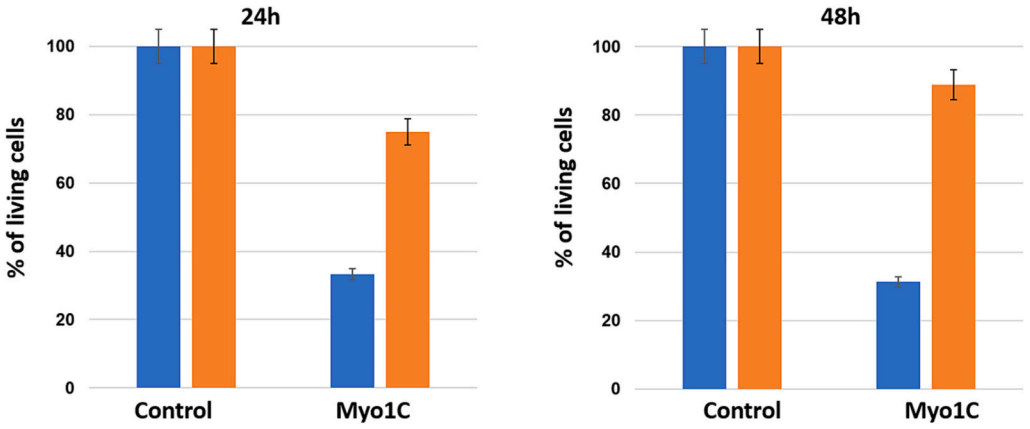

Fig. 5. Time-dependent inhibitory effect of the 48/Myo1C on the viability (MTT-test) of anti-CD3-activated lymphocytes of human peripheral blood. Control – untreated cells; Myo1C – cells treated with the 48/Myo1C (0.02 mg/ml). Blue column - anti-CD3 positive, brown column - anti-CD3-negative cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

serum of the severe Covid-19 patients, followed by a "steady" phase characterized by only moderately increased level of the Myo1C in blood serum of the Covid-19 patients. Indirectly, our hypothesis about the existence of the "acute" and "steady" phases in time dynamics of the 48/Myo1C content in blood serum of the autoimmune patients, including Covid-19 patients, is in agreement with the results of our recent clinical investigation of the correlation between the level of the 48/Myo 1C in blood serum of the multiple sclerosis (MS) patients and the clinical status of this disease. We have demonstrated that higher level of the 48/Myo1C in blood serum of the MS patients was associated with an early stage of this autoimmune disease characterized by its short duration and the relapsing-remitting type (Nehrych et al., 2018). By the way, the exacerbations of the disease in the MS patients were cured with the dexamethasone (Berkovich, 2013). Thus, the Myo1C may participate in the mediation of the immune suppressive effects of the Dexamethasone in the severe Covid-19 patients, and, possibly, in patients with other autoimmune diseases.

The application of the affinity chromatography on the 48/Myo1C-functionalized magnetic microspheres in combination with MALDI-TOF mass-spectrometry and molecular modeling *in silico* allowed us to identify two more potential protein partners capable of interacting with the 48/Myo1C. We revealed that 48/Myo1C possessed an affinity to the component 3 of the immune complement system and the anti-thrombin III participating in blood coagulation cascade (Starykovych et al., 2021). The component 3 is a protein of blood serum playing a central role in the complement system and contributing to an innate immunity (Sahu and Lambris, 2001). Besides, the C3 component can clear up the pathogens and it is also important for a variety of homeostatic processes (tissue regeneration and synapse pruning), as well as for clearing debris and controlling tumor cell progression (Ricklin et al., 2016). The isoform 1 of the anti-thrombin III is a protease inhibitor present in human blood plasma. It belongs to serpin superfamily and inhibits thrombin functions, as well as the action of other activated serine proteases of the coagulation cascade, also demonstrating potent anti-inflammatory properties (Myronovskij et al., 2017). Thus, a capability of the 48/Myo1C to affect functional activity of specific proteins of the complement (component 3) and coagulation (anti-thrombin-III) cascades, as well as its role in a suppression of the viability of lymphocytes, may be critically important in Covid-19 pathogenesis, especially at its severe forms.

4. Conclusions

Here, we firstly demonstrated that blood serum of the Covid-19 patients with severe disease manifestation contains the elevated amount of 48 kDa protein soluble in 10% TCA. By means of the MALDI–TOF mass-spectrometry and bioinformatic analysis, this protein was identified as the truncated form of the unconventional 48/Myo 1C. It was found that dexamethasone treatment (24–72 h) of the severe Covid-19 patients led to an increase in content of the 48/Myo1C in their blood serum, while at prolongation of such treatment to 96 h, its content was significantly decreased. The 48/Myo 1C time-dependently inhibited the viability of the lymphocytes activated with the anti-CD3 antibodies. The products of degradation of intact Myo1C were detected by electrophoresis of the lysate of human peripheral blood lymphocytes induced to apoptosis by the UV-radiation. These products were identified with the Western-blot analysis using monospecific anti-Myo1C antibodies.

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